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(54) Title: RAT OB RECEPTORS AND NUCLEOTIDES ENCODING THEM

(57) Abstract

The rat *ob* receptor gene has been isolated and cloned. Two different alleles have been identified: the wild-type, and the *fa*-allele which differs from the wild-type by only one base pair. The base pair change, however, introduces an *Msp*I restriction site into the DNA sequence, and also results in an amino acid change. Also part of the invention are the novel receptors, vectors containing the nucleic acid encoding the receptors, host cells transformed with this gene, and assays which use the gene or protein and identify new ligands.

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TITLE OF THE INVENTION
RAT *OB* RECEPTORS AND NUCLEOTIDES ENCODING THEM

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of Provisional patent application Serial No. _____, (Attorney Docket No. 19642PV) filed February 22, 1996, which is hereby incorporated by reference.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not Applicable

REFERENCE TO MICROFICHE APPENDIX

Not Applicable

FIELD OF THE INVENTION

This invention relates to rat *ob* receptor proteins, to DNA and RNA sequences encoding them, and to assays using rat receptor proteins.

BACKGROUND OF THE INVENTION

Recently the identification of mutations in several genes involved in the onset of obesity in rodents have been identified. Of particular interest are mutations discovered in the peptide hormone, leptin, which is a component of a novel signal transduction pathway that regulates body weight (Zhang *et al.* 1994, *Nature* 372:425-432; Chen *et al.* 1996, *Cell* 84:491-495). Leptin was initially discovered by the positional cloning of the obesity gene, *ob*, in mice. Two different ob alleles have been identified: one mutation causes the premature termination of the leptin peptide resulting in a truncated protein, and the other mutation changes the transcriptional activity of the *obesity* (*ob*) gene, resulting in a reduced amount of circulating leptin.

There is a correlation between a decrease in the levels of biologically active leptin and the overt obese phenotype observed in *ob/ob* mice. Recombinant leptin has been shown to induce weight loss in

the *ob/ob* mouse but not in the diabetic phenotype *db/db* mouse (Campfield *et al.* 1995, *Science* 269: 546-549; Halaas *et al.* 1995, *Science* 269: 543-546; Pellymounter *et al.* 1995, *Science* 269:540-543; Rentsch *et al.* 1995, *Biochem. Biophys. Res. Comm.* 214:131-136; and Weigle *et al.* 1995, *J. Clin. Invest.* 96:2065-2070).

Although the synthesis of leptin occurs in the adipocyte, its ability to decrease food intake and increase metabolic rate appears to be mediated centrally by the hypothalamus. Injection of recombinant leptin into the third ventricle of the brain elicits a similar response as peripheral administration of leptin. Furthermore, the recent cloning of the human receptor for the leptin, the ob-receptor (OB-R), reveals that it is transcribed in the hypothalamus (Tartaglia *et al.* 1995, *Cell* 83:1263-1271; Stephens *et al.* 1995, *Nature* 377: 530-532). In addition, a mutation that results in premature termination of the long-form of the mouse OB-R, which is preferentially expressed in the hypothalamus, appears to be responsible for the obese phenotype of the *db/db* mouse (Lee *et al.* 1996, *Nature* 379:632-635; Chua *et al.* 1996, *Science* 271:994-996; and Chen *et al.* 1996, *Cell* 84:491-495).

The *fa* mutation is a recessive allele that arose spontaneously in the 13M rat strain and was first reported in 1961 (Zucker *et al.* 1961, *J. Heredity* 52: 275-278. The onset of obesity in the *fa/fa* Zucker rat is at 5-7 weeks of age and progresses with age. The mature fatty rat is approximately twice the weight of lean litter mates and over 40% of its body weight is adipose tissue (Zucker *et al.* 1962, *Proc. Soc. Exp. Biol. Med.* 110:165-171; Zucker *et al.* 1963, *J. Nutrition* 80:6-19). The *fa/fa* Zucker rat exhibits hypercholesterolemia, hyperlipemia, and hyperglycemia and has been used extensively as an animal model for human cardiovascular disease and diabetes. Most of the fatty Zucker rat colonies have been maintained by outbreeding in order to retain heterozygousity at as many loci as possible. However, certain stocks have been inbred to produce animals such as the Zucker diabetic fatty (ZDF) rat which exhibits a more profound diabetic phenotype than the outbred *fa/fa* Zucker rat (Clark, *et al.* 1983, *Proc. Soc. Exp. Biol. Med.* 173: 68-75).

The *fa* mutation maps to rat chromosome 5 in a region that is syntenic with the *db* allele on mouse chromosome 4 (Truett, *et al.* 1991, *Proc. Natl. Acad. Sci.* 88: 7806-7809). This observation, in conjunction with the similar phenotypes of the *fa/fa* rat and the *db/db* mouse, led to the proposal that the *fa* gene was the rat homologue of the *db* gene. Higher resolution genetic mapping supports the contention that the *fa* mutation is located in the gene encoding the rat OB-R (Chua *et al. Science* 271: 994).

It would be desirable to be able to further experiment with the rodent model system for obesity, and to be able to clone and produce purified rat *ob* receptor to use in assays for the identification of ligands which may be useful in understanding obesity and for its prevention and treatment.

SUMMARY OF THE INVENTION

Not Applicable

BRIEF DECRIPTION OF THE DRAWINGS

FIGURE 1 is the amino acid sequence of the rat OB-receptor.

FIGURE 2 is the cDNA sequence of the rat OB-receptor.

FIGURE 3 is a table of primers used for the PCR reactions detailed in the Examples.

FIGURE 4 shows the gels demonstrating the analysis of the A^{880} to C mutation identified in the OB-receptor from hypothalamic cDNA and genomic DNA obtained from lean and *fa/fa* rats.

FIGURE 5 compares the amino acid sequence between human cytokine receptor gp130 (Humgp 130), the mouse OB-R (MousOBR), human OB-R (HumOBR) and lean rat OB-R (RatOBR). The numbering refers to the location in the protein, and the cytokine motif GXWSXWS can be seen.

As used througout the specification and claims, the following definitions apply:

"Substantially free from associated rat membrane proteins" means that the rat receptor protein is not in physical contact with any rat membrane proteins.

"Substantially purified rat OB-receptor" means that the rat receptor protein is at least 90% and preferably at least 95% pure.

"Wild type" means that the gene or protein is substantially the same as that found in a rat which is not considered to have a mutation for that gene or protein. It is also referred to as "lean" throughout the specification and claims.

"*fa*" means that the gene or protein is substantially the same as that found in a rat homologous for the *fatty* mutation.

"Substantially the same" when referreing to a nucleic acid or amino acid sequence means either it is the same as the reference sequence, or if not exactly the same, contains changes which do not affect its biological activity or function. Although the *fa* and wild type rat OB-R genes differ by only one nucleotide, they are not considered "substantially the same" as the biological activity and functions of their encoded proteins are very different.

The rat OB-R is a member of the cytokine receptor family. Motifs that are characteristic of the cytokine receptors such as the motif WSXWS (where W is the amino acid residue tryptophan, S is the amino acid residue serine and X is any amino acid.) were found to be conserved in the rat OB-R.

One aspect of this invention is the molecular cloning of a rat OB-R. The nucleotide sequence for the rat OB-R from both lean and *fa/fa* rat hypothalamic cDNA was determined and compared. In the *fa/fa* rat, there was a single nucleotide change, an A to C at nucleotide 880 resulting in an amino acid change at glutamine 269 to proline. The mutation introduces an *Msp* I site (CCGG) that was utilized to genotype a number of lean control and *fatty* animals. The results indicate that the mutation is tightly linked to the *fa* allele. Thus, it is likely that the *fa* mutation lies in the OB-R receptor cDNA and that the A to C

transversion at base pair 880 is responsible for the obese phenotype. Both rat OB-R alleles, i.e. the OB-R containing a glutamine 269 and the allele containing proline 269 are part of this invention, as are all nucleic acids which can encode them.

The nucleotide sequence of the wild type rat OB-R cDNA obtained in accordance with this invention has 3650 nucleotides, as shown in FIGURE 2. This DNA sequence contains an open reading frame from nucleotide 75 to 3653 that encodes a protein of 1162 amino acids. The open reading frame extending from nucleotide 75 to 3653 makes up one aspect of this invention.

The wild type and *fa* receptor proteins contain an extracellular, a transmembrane domain. The extracellular domain extends from amino acids 1-830; the transmembrane domain is from amino acids 839-860; and the cytoplasmic domain is from amino acids 860-1162. This invention also includes proteins which lack one or more of these domains. Such deleted proteins are useful in assays for identifying ligands and their binding activity.

It has also been found that alternate splicing can occur in the receptor gene processing. This can occur at base pair 2742 ($lysine^{889}$). The alternative sequence (for both the wild type and *fa*) genes and receptors, is shown below and forms another aspect of this invention:

AGA	GCG	GAC	ACT	CTT	TGA	ATA	TCT
R	A	D	T	L	STOP		

Amino acids 1-28 form a signal sequence; thus the mature proteins extend from amino acids 28-1162. The mature proteins form yet another aspect of this invention. This differs from the signal sequence of 1-22 reported for mouse and human OB-r; this may be explained by the use of a different analysis program.

Comparison of wild type rat OB-R to known OB-R receptors of different species has revealed some similarities. For example, the rat OB-R nucleotide sequence is 93% identical to the

mouse OB-R and 81% identical to the human OB-R sequences. The deduced amino acid sequence of the rat OB receptor is 93% identical to the mouse and 76% identical to the human OB-R.

The size of the open reading frame of the rat OB-receptor of this invention, (1162 amino acids) is similar to that of the human OB-R (1165 amino acids) reported by Toriaglla et al. 1995, *Cell* 83:1-20. Both the rat OB-R of this invention and the human OB-R contain a large cytoplasmic domain. In contrast, the mouse OB-receptor of 894 amino acids has a relatively short cytoplasmic domain.

One of the most notable and surprising aspects of this invention is that there is only a single nucleotide difference between the wild type rat cDNA and the *fa/fa* rat cDNA for the OB-R. PCR fragments obtained from *fa/fa* cDNA were sequenced. A single nucleotide change relative to the lean cDNA sequence was observed in the hypothalamus. An A to C transversion at bp 880 results in an amino acid change of glutamine to proline at amino acid residue 268. Every tissue examined in the *fa/fa* rat was found to be homozygous for this A to C mutation at nucleotide 880. The A to C change in the sequence introduces a MspI restriction endonuclease site (CCGG) into the sequence, and this is the basis of an assay for presence of the mutation.

Thus another aspect of this invention is an assay to determine the genotype of a OB-R DNA, suspected of having an A to C mutation at bp 880, comprising digesting the OB-R DNA with *Msp*I, and comparing the restriction products so producted. In a preferred embodiment, the assay comprises generating PCR products of the OB-R DNA, digesting the PCR products with *Msp*I, and comparin the restriction products so produced with those obtained from at containing a wild-type OB-R gene. The gene from a rat which has a wild-type OB-R will yield two restriction products, 1774 and 289 bp long. The gene from the *fa* rat will have three restriction products: 747, 1027 and 289 bp long. These are easily observed using standard gel techniques.

The OB-R gene can be introduced into virtually any host cell using known vectors. Preferred host cells include *E. coli* as well as mammalian and yeast cell lines.

One of ordinary skill in the art is able to choose a known vector which is appropriate for a given host cell; generally plasmids or viral vectors are preferred. The OB-R gene may be present in the vector in its native form, or it may be under the control of a heterologous promoter, and if desired, one or more enhancers, or other sequences known to regulate transcription or translation. The host cell containing the OB-R gene is cultured, and the OB-R gene is expressed. After a suitable period of time the OB-R protein may be harvested from the cell using conventional separation techniques.

A further aspect of this invention is the use of rat OB-R in assays to identify OB-R ligands. A ligand binds to the OB-R, and *in vivo* may or may not result in an activation of the receptor. Ligands may be agonists of the receptor (i.e. stimulate its activity), antagonists (inhibit its activity) or they may bind with little or no effect upon the receptor activity.

In an assay for ligands, the rat OB-R of this invention is exposed to a putative ligand, and the amount of binding is measured. The amount of binding may be measured in many ways; for example, a ligand or the OB-R being investigated may be labeled with a conventional label (such as a radioactive or fluorescent label) and then put in contact with the OB-R under binding conditions. After a suitable time, the unbound ligand is saparated from the OB-R and the amount of ligand which has bound can be measured. This can be performed with either the wild-type OB-R or the *fa* OB-R of this invention; alternatively the amount of binding to the two alleles can be compared. In a competitive assay, both the putative ligand and a known ligand are present, and the amount of binding of the putative ligand is compared to the amount of binding to a known ligand. Alternatively, the putative ligand's ability to displace previously bound known ligand (or vice-versa) may be measured. In yet other embodiments, the assay may be a heterogeneous one, where the OB-R may be bound to a surface, and

contacted with putative ligands. Dectection of binding may be by a variety of methods, including labelling, reaction with antibodies, and chomophores.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to a rat *ob* receptor which is substantially free from associated rat membrane proteins. It also relates to substantially purified rat *ob* receptor ("rat OB-R" or "rat OB-receptor") protein. One of the rat OB-Rs of this invention is obtained from a rat which has a wild-type OB-R. Another rat OB-R of this invention is obtained from a rat which has the *fa* mutation.

Another aspect of this invention is to nucleic acids which encode a rat OB receptor. The nucleic acid may be any nucleic acid which can encode a protein, such as genomic DNA, cDNA, or any of the various forms of RNA. Preferably, the nucleic acid is cDNA.

This invention also includes vectors containing a rat OB-R gene, host cells containing the vectors, and methods of making susbstantially pure rat OB-R protein comprising the steps of introducing a vector comprising a rat OB-R gene into a host cell, and cultivating the host cell under appropriate conditions such that rat OB-R is produced. The rat OB-R so produced may be harvested from the host cells in conventional ways.

Yet another aspect of this invention are assays which employ a rat OB-R. In these assays, various molecules, suspected of being rat OB-R ligands are contacted with a rat OB-R, and their binding is detected. In this way agonists, antagonists, and ligand mimetics may be identified. A further aspect of this invention are the ligands so indentified.

The following non-limiting Examples are presented to better illustrate the invention.

EXAMPLE 1

Preparation of mRNA and cDNA from rat tissues

Tissues were collected from lean and *fa/fa* Zucker rats and snap frozen in liquid nitrogen. The tissues collected included: hypothalamus, pituitary, lung, liver, kidney, heart, adrenal glands, smooth muscle, skeletal muscle, and adipose tissue. The tissues were homogenized with a Brinkmann Polytron homogenizer in the presence of guanadinium isothiocyanate. mRNA was prepared from hypothalamus, lung, and kidney according to the instructions provided with the messenger RNA isolation kit (Stratagene, La Jolla, CA). cDNA was prepared from approximately 2 µg of mRNA with the SuperScript™ choice system (Gibco/BRL Gaithersburg, MD). The first strand cDNA synthesis was primed using 1 ug of oligo(dT)12-18 primer and 25 ng of random hexamers per reaction. Second strand cDNA sythesis was performed according to the manufacturer's instructions. The quality of the cDNA was assessed by labeling an aliqout (1/10th) of the second strand reaction with approximately 1 µCi of [a-^{32}P]dCTP (3000 Ci/mmol). The labeled products were separated on an agarose gel and detected by autoradiography.

EXAMPLE 2

Amplification of Lean Rat OB-receptor cDNA using PCR

The initial portion of the rat OB receptor was obtained by PCR using degenerate primers based on the mouse and human OB-receptor amino acid sequences. A set of 9 oligonucleotide primers, ROBR 1-9, shown in FIGURE 3, were designed to regions with low codon degeneracy. The pairing of the forward primers ROBR 2 (5'-CAY TGG GAR TTY CTI TAY GT-3') and ROBR 3 (5'-GAR TGY TGG ATG AAY GG-3') corresponding to mouse amino acid sequences

HWEFLYV and ECWMKG, with reverse primers ROBR 6 (5 '-ATC CAC ATI GTR TAI CC-3'), 7(5'-CTC CAR TTR CTC CAR TAI CC-3'), and 8 (5'-ACY TTR CTC ATI GGC CA-3') representing mouse amino acids, GYTMWI, VYWSNWS, and WPMSKV provided good yields of the appropriately sized products. The fragments of interest were amplified as long polymerase chain reaction (PCR) products by a modifying the method of Barnes (1994, *Proc. Natl. Acad. Sci.* 91:2216-2220, which is hereby incorporated by reference. In order to obtain the required long PCR fragments, Taq Extender (Stratagene, La Jolla CA.) and the Expand Long Template PCR System (Boehringer Mannheim, Indianapolis, IN) were used in combination. The standard PCR reaction mix, in a final volume of 20 µl, contained 5 ng of template (lean rat cDNA), 100 ng of primers, 500 µM dNTPs, 1 X Buffer 3 from the Expand kit, 0.1 µl each of Taq Polymerase and Taq Expander. Reactants were assembled in thin walled reaction tubes. The amplification protocol was1 cycle of 92°C for 30 sec., followed by 32 cycles at 92°C for 30 sec., 45°C for 1 min. and 68°C for 3 min. using a Perkin-Elmer (Norwalk, CT) 9600 Thermal Cycler.

This strategy produced a series of PCR products with the largest being approximately 2.2 Kbp amplified from primers ROBR 2 and ROBR 8. These products were subcloned for DNA sequence analysis as described below.

EXAMPLE 3

Subcloning of PCR products

PCR products of the appropriate size were prepared for subcloning by separation on an agarose gel, excising the band, and extracting the DNA using Prep-A-Gene (BioRad, Richmond, CA). PCR products were ligated into pCR™II (Invitrogen, San Diego, CA) according to the instructions provided by the manufacturer. The ligation was transformed into INVaF' cells and plated on Luria-Bertani plates containing 100 µg/ml ampicillin and X-Gal (32 µl of 50 mg/ml X-Gal (Promega, Madison, WI). White colonies were picked and grown

overnight in Luria -Bertani broth plus 100 µg/ml ampicillin. Plasmid DNAs were prepared using the Wizard miniprep kit (Promega, Madison, WI). Inserts were analyzed by digesting the plasmid DNA with EcoRI and separating the restriction endonulease digestion products on an agarose gel.

Plasmid DNA was prepared for DNA sequencing by ethanol precipitation and resuspending in water to achieve a final DNA concentration of 100 µg/ml. DNA sequence analysis was performed using the ABI PRISM™ dye terminator cycle sequencing ready reaction kit with AmpliTaq DNA polymerase, FS. The initial DNA sequence analysis was performed with M13 forward and reverse primers, subsequently primers based on the rat OB-R sequence were utilized. Following amplification in a Perkin-Elmer 9600, the extension products were purified and analyzed on an ABI PRISM 377 automated sequencer (Perkin Elmer, Norwalk, CT). DNA sequence data was analyzed with the Sequencher program. Due to the unknown genotype of the lean Zucker rat for the *fa* allele, either (+/+ or +/*fa*) the DNA sequence of multiple subclones of each fragment was analyzed to determine the cDNA sequence of the lean rat OB-R.

EXAMPLE 4

Amplification and DNA sequence analysis of lean and *fa/fa* with primers ROBR 10 and 17

Once specific lean rat sequence had been obtained from the ROBR 2-8 PCR fragment, rat specific primers ROBR 10 (5'-CTG CAC TTA ACC TGG CCT ATC-3') and ROBR 17 (5'-GGC CAG AAC TGT AAC AGT GTG-3') were synthesized. Using primers ROBR 10 and 17, PCR products were amplified from rat lean hypothalamus, lean lung, *fa/fa* hypothalamus and *fa/fa* kidney cDNAs. The PCR conditions used for this reaction were a PCR reaction mix with a total volume of 50 µl containing 5 ng of template (various rat cDNAs mentioned above), 200 ng of primers. 500 µM dNTPs, 1 X Buffer 3 from the Expand kit, 0.25 µl each of Taq Polymerase and Taq Expander. Reactants were

assembled in thin walled reaction tubes. The amplification protocol was 1 cycle of 92°C for 30 sec., followed by 32 cycles at 92°C for 30 sec., 60°C for 1 min. and 68°C for 4 min. using a Perkin Elmer 9600 Thermal Cycler.

EXAMPLE 5

Amplification of the 3' portion of the rat OB-R cDNA using Semi-nested PCR

The 3' end of both the lean and *fa/fa* rat OB-receptors was obtained by the PCR with an initial amplification of the rat cDNA using a rat specific 5' primer paired with either a degenerate primer that corresponds to the cytoplasmic domain of the human OB-receptor or the 3' UTR of the human or mouse sequences. This was followed by a second short round of amplification with either one of the original primers paired with a nested primer positioned within the originally amplified fragment, or with two nested primers.

Rat specific primers ROBR 15 (5'-TCA CCT TGC TTT GGA AGC C-3'), ROBR 16 (5'-GAC ATG GTC ACA AGA TGT GGG-3') and ROBR 23 (5'-CCT GGA CAC TGT CAC CTG ATG-3') were paired in different combinations with human degenerate primers located in the cytoplasmic domain of the human OB receptor; HOBR 5 (5'-CAT CAT YTC RTC YTT RTT YTT CCA-3'), HOBR 6 (5'-GTY TGR AAY TGI GGC AT-3') and HOBR 7 (5'-TCR CAC ATY TTR TTY TCC AT-3') which correspond to amino acids WKNKDEMM, MPQFQT, and MENKMCD, respectively. Primers from the 3' ends of the human, HOBR 1R (5'-TCT CTC CCA CCC ACA ACT AT-3'), and mouse, MOBR 1R (5'-TGG GTT CAT CTG TAG TGG TC-3'), OB receptors were also paired with rat specific primers.

PCR reactions were performed with various combinations of the above primer sets in a total volume of 20 µl containing 5 ng of template (lean and *fa/fa* hypothalamus cDNAs), 100 ng of primers, 500 µM dNTPs, 1 X Buffer 3 from the Expand kit, 0.1 µl each of Taq Polymerase and Taq Expander. Reactants were assembled in thin walled

reaction tubes for the Perkin Elmer 9600 Thermal cycler. The amplification protocol was 1 cycle of 92°C for 30 sec., followed by 32 cycles at 92°C for 30 sec., 45°C for 1 min. and 68°C for 4 min. using a Perkin Elmer 9600 Thermal Cycler.

Products were then purified, removing all nucleotides and primers, using the QIAquick PCR purification kit according to the manufacturer's specified protocols and resuspended in 30 µl of water. The second PCR step was then performed using the first PCR reaction as the template and a nested rat specific primer paired with the original 3' primer as outlined above. The reaction conditions were a 50 µl reaction containing 5 µl of template (from the purified PCR product), 200 ng of primers, 500 µM dNTPs, 1 X Buffer 3 from the Expand kit, 0.25 µl each of Taq Polymerase and Taq Expander. Reactants were assembled in thin walled reaction tubes for the Perkin Elmer 9600 Thermal cycler. The amplification protocol was 1 cycle of 92°C for 30 sec., followed by 25 cycles at 92°C for 30 sec., 45°C for 1 min. and 68°C for 4 min. using a Perkin Elmer 9600 Thermal Cycler.

The largest fragment that was generated using the strategy was a fragment produced from ROBR 16 and HOBR 1R that was approximately 1500 bp in length. The mouse 3' UTR which presumably encodes a smaller isoform generated by alternative splicing, produced a fragment that was about 650 bp long.

EXAMPLE 6

Amplification of 5' end of the rat OB receptor

The 5' end of the rat OB receptor was obtained by using semi-nested PCR in a manner analogous to that described above for the 3' end. In this case the rat specific primers are the 3' primers that were combined with primers from the 5' UTRs of the human OB-receptor. The primers utilized were HOBR 1F (5'-CTT ATG CTG GGA TGT GCC-3') and HOBR 1F-2 (5'-TCG TGG CAT TAT CCT TCA G-3') paired with either ROBR 11 (5'-GAT AGG CCA GGT TAA GTG CAG-3') or ROBR 12 (5'-GAG TGC GGA GCA GTT TTG AC-3).

The largest product, HOBR 1F-2 and ROBR 11, yielded a 500 bp fragment that covers the region and incluces an initiator methionine codon.

EXAMPLE 7

Identification of a nucleotide change in the *fa/fa* cDNA

PCR fragments obtained from *fa/fa* cDNA were prepared for DNA sequence analysis by separating the PCR products on an agarose gel, excising the band of interest, and extracting the DNA using Prep-A-Gene (BioRad). Sequencing results of the PCR product generated from *fa/fa* hypothalamic cDNA identified a single nucleotide change relative to the lean cDNA sequence. An A to C transversion at bp 880 results in an amino acid change of glutamine to proline at amino acid residue 268. The A to C change in the sequence introduces a MspI restriction endonuclease site (CCGG) into the sequence.

Several independent PCR products were amplified from hypothalamus, lung and kidney cDNA from lean and *fa/fa* tissues using the primer pair ROBR 10 and 17. This product contains only one endogenous *Msp* I site at nucleotide 1907. Restriction digestion of the PCR products in a reaction that consisted of 5 µl of the PCR reaction, 4 µl of water and 1 µl of the restriction endonuclease *Msp* I. These were mixed, incubated for 1 hr at 37°C and analyzed on a 1% agarose gel. The PCR products from the lean rat cDNAs contained only the endogenous *Msp* I site and generated products of 1774 and 289 bp. In contrast the PCR products from the *fa/fa* cDNAs contained an additional *Msp* I site identified during the sequencing of ROBR 10/17 and generated products of 747, 1027, and 289. Thus, every tissue examined in the *fa/fa* rat was homozygous for the A to C mutation at nucleotide 880.

EXAMPLE 8

Genotype analysis of lean and *fa/fa* rats

Genomic DNA was prepared from a 2 cm portion of the tail from ten lean and ten *fa/fa* Zucker rats and 2 lean and 5 *fa/fa* ZDF rats. The tissue was digested overnight at 55°C using 0.3 µg of Proteinase K in 0.7 ml buffer containing 50 mM Tris, pH 8.0, 100 mM EDTA, and 0.5% SDS. The DNA was extracted two times with phenol/chloroform and one time with chloroform. The DNA was precipitated by adding NaCl to achieve a concentration of 0.3M and then adding an equal volume of 100% ethanol. The DNA was transferred to a 70% wash and then resuspended in 10 mM Tris, 1 mM EDTA.

Genomic DNA, obtained as outlined above from various sources, was diluted in water to a final concentration of approximately 100 ng/ul. In this experiment, the reaction conditions were a 20 µl reaction containing 1 µl of genomic DNA template, 100 ng of primers, 500 µM dNTPs, 1 X Buffer 3 from the Expand kit, 0.25 µl each of Taq Polymerase and Taq Expander. Reactants were assembled in Perkin Elmer 0.5 ml thin walled reaction tubes. The amplification protocol for a Perkin Elmer 480 Thermal Cycler was 32 cycles of 92°C for 30 sec., 54°C for 1 min. and 68°C for 5 min. Primers ROBR 27 (5'-GTT TGC GTA TGG AAG TCA CAG-3') and ROBR 28 (5'-ACC AGC AGA GAT GTA TCC GAG-3') were used to amplify a 1.8 Kbp fragment that must contain approximately 1.65 Kbp of intronic sequence since these primers only produce a 156 bp PCR fragment when amplifying cDNA.

After PCR amplification, an *Msp* I restriction endonuclease digestion of the products was undertaken. The reaction contained 5 µl of the PCR reaction, 4 µl of water and 1 µl of the restriction endonuclease *Msp* I. These were mixed and incubated for 1 hr at 37°C. The products were then analyzed on a 1% agarose gel. The PCR products contained an endogenous *Msp* I site that cleaves the fragment somewhere in the intron and produces a 700 bp fragment. Thus, the *Msp* I restriction endonuclease digestion of the 1800 bp ROBR 27/28 PCR product from a homozygous lean rat yields two fragments of 1100

bp and the endogenous 700 bp fragment. In contrast, *Msp* I digestion of PCR products from a *fa/fa* ROBR 27/28 PCR amplification, which contains the A to C mutation, introduces an additional *Msp* I site that cleaves the 1100 bp band to produce a 950 bp and a small fragment of 130 bp. The genomic analysis of the lean Zucker and ZDF rats also demonstrated that Fa/*fa* heterozygotes where present as illustrated by *Msp* I restriction endonuclease digestion patterns that showed that these rats had the 1100 bp fragments as well as the 950 mutant fragment.

WHAT IS CLAIMED IS:

1. A rat *ob*-receptor (OB-R), sustantially free from associated rat proteins.

2. A rat OB-R according to Claim 1 which is substantially pure.

3. A rat OB-R according to Claim 1 which is from a rat which has a wild-type OB-R.

4. A rat OB-R according to Claim 1 which is from a rat having an *fa* OB-R.

5. An OB-R according to Claim 3 which is shown in FIGURE 1.

6. A nucleic acid encoding a rat OB-R of Claim 1.

7. A nucleic acid according to Claim 6 which is a DNA.

8. A nucleic acid according to Claim 7 which is shown in FIGURE 2.

9. A nucleic acid according to Claim 7 which encodes the ORF from from nucleotide 75 to 3653 as depicted in FIGURE 1.

10. A DNA encoding substantially purified *fa* OB-R.

11. A vector comprising a nucleic acid which encodes a rat OB-R.

12. A vector according to Claim 11 which is a plasmid.

13. A vector according to Claim 12 which is a viral vector.

14. A host cell containing a vector according to Claim 11.

15. A host cell according to Claim 14 which is *E. coli*, a mammalian cell, or a yeast cell.

16. An assay to determine whether a rat OB-R gene is wild-type or an *fa* allele, comprising: replicating PCR primers from the gene; cutting the primers with *Msp*I restriction enzyme; and determining the length of the resulting fragments.

17. An assay to determine if a putative ligand binds to a rat OB-R and an assay for binding putative ligands to the *fa*-OB-R comprising: contacting the putative ligand with a rat OB-R, and determining if binding has occurred.

18. An assay according to Claim 17 wherein the ligand is labeled.

19. An assay according to Claim 17 wherein the rat OB-R is labeled.

20. A ligand identified by the assay of Claim 17.

```
  1  MTCQKFYVVL LHWEFLYVIT ALNLAYPTSP WRFKLFCAPP STTDDSFLSP
 51  AGVPNNTSSL KGASEALVEA KFNSTGIYVS ELSKTIFHCC FGNEQGQNCS
101  ALTGNTEGKT LASVVKPLVF RQLGVNWDIE CWMKGDLTLF ICHMEPLLKN
151  PFKNYDSKVH LLYDLPEVID DLPLPPLKDS FQTVQCNCSV RECECHVPVP
201  RAKVNYALLM YLEITSAGVS FQSPLMSLQP MLVVKPDPPL GLRMEVTDDG
251  NLKISWDSQT KAPFPLQYQV KYLENSTIVR EAAEIVSDTS LLVDSVLPGS
301  SYEVQVRSKR LDGSGVWSDW SLPQLFTTQD VMYFPPKILT SVGSNASFCC
351  IYKNENQTIS SKQIVWWMNL AEKIPETQYN TVSDHISKVT FSNLKATRPR
401  GKFTYDAVYC CNEQACHHRY AELYVIDVNI NISCETDGYL TKMTCRWSPS
451  TIQSLVGSTV QLRYHRRSLY CPDNPSIRPT SELKNCVLQT DGFYECVFQP
501  IFLLSGYTMW IRINHSLGSL DSPPTCVLPD SVVKPLPPSN IKAEITINTG
551  LLKVSWEKPV FPENNLQFQI RYGLNGKEIQ WKTHEVFDAK SKSASLPVSD
601  LCAVIAVQVR CRRLDGLGYW SNWSSPAYTL VMDVKVPMRG PEFWRIMDGD
```

FIG.1A

```
651  ITKKERNVTL LWKPLMKNDS LCSVRRYVVK HRTAHNGTWS QDVGNQTNLT
701  FLWAESAHTV TVLAINSIGA SLVNFNLTFS WPMSKVNAVQ SLSAYPLSSS
751  CVILSWTLSP NDYSLLYLVI EWKNLNDDDG MKWLRIPSNV NKYYIHDNFI
801  PIEKYQFSLY PVFMEGVGKP KIINGFTKDD IAKQQNDAGL YVIVPIIISS
851  CVLLLGTLLI SHQRMKKLFW DDVPNPKNCS WAQGLNFQKP ETFEHLFTKH
901  AESVIFGPLL LEPEPVSEEI SVDTAWKNKD EMVPAAMVSL LLTTPDSTRG
951  SICISDQCNS ANFSGAQSTQ GTCEDECQSQ PSVKYATLVS NVKTVETDEE
1001 QGAIHSSVSQ CIARKHSPLR QSFSSNSWEI EAQAFFLLSD HPPNVISPQL
1051 SFSGLDELLE LEGNFPEENH GEKSVYYLGV SSGNKRENDM LLTDEAGVLC
1101 PFPAHCLFSD IRILQESCSH FVENNLNLGT SGKNFVPYMP QFQSCSTHSH
1151 KIIENKMCDL TV
```

FIG.1B

```
  1  TGGGGCAATT GGGCTGACCT TTCTTATGCT GGGATGTGCC TTGGAGGACT
 51  ATGGGTGTCT ATCTCTGAAG TAAGATGACG TGTCAGAAAT TCTATGTGGT
101  TTTGTTACAC TGGGAATTTC TGTATGTGAT AACTGCACTT AACCTGGCCT
151  ATCCAACCTC TCCCTGGAGA TTTAAGCTGT TTTGTGCGCC ACCGAGTACA
201  ACTGATGACT CCTTTCTCTC TCCTGCTGGA GTCCCAAACA ATACTTCGTC
251  TTTGAAGGGG GCTTCTGAAG CACTTGTTGA AGCTAAATTT AATTCAACTG
301  GTATCTACGT TTCTGAGTTA TCCAAAACCA TTTTCCACTG TTGCTTTGGG
351  AATGAGCAAG GTCAAAACTG CTCCGCACTC ACAGGCAACA CTGAAGGGAA
401  GACGCTGGCT TCAGTGGTGA AGCCTTTAGT TTTCCGCCAA CTAGGTGTAA
451  ACTGGGACAT AGAGTGCTGG ATGAAAGGGG ACTTGACATT ATTCATCTGT
501  CATATGGAAC CATTACTTAA GAACCCCTTC AAGAATTATG ACTCTAAGGT
551  TCACCTTTTA TATGATCTGC CTGAAGTTAT AGATGATTTG CCTCTGCCCC
601  CACTGAAAGA CAGCTTTCAG ACTGTCCAGT GCAACTGCAG TGTTCGGGAA
```

FIG.2A

```
 651  TGCGAATGTC ATGTACCAGT ACCCAGAGCC AAAGTCAACT ACGCTCTTCT
 701  GATGTATTTA GAAATCACAT CTGCTGGTGT GAGTTTTCAG TCACCTCTAA
 751  TGTCACTGCA GCCCATGCTT GTTGTGAAGC CCGATCCACC GCTGGGTTTG
 801  CGTATGGAAG TCACAGATGA TGGTAATTTA AAGATTTCAT GGGACAGCCA
 851  AACAAAAGCA CCATTTCCAC TTCAATATCA GGTGAAATAT TTAGAGAATT
 901  CTACAATCGT AAGAGAGGCT GCTGAAATCG TCTCGGATAC ATCTCTGCTG
 951  GTAGACAGCG TGCTTCCTGG GTCTTCATAC GAGGTCCAGG TGAGGAGCAA
1001  GAGACTGGAT GGCTCAGGAG TCTGGAGTGA CTGGAGTTTA CCTCAACTCT
1051  TTACCACACA AGATGTCATG TATTTTCCAC CCAAAATTCT GACGAGTGTT
1101  GGATCCAATG CTTCCTTTTG CTGCATCTAC AAAAATGAGA ACCAGACTAT
1151  CTCCTCAAAA CAAATAGTTT GGTGGATGAA TCTAGCCGAG AAGATCCCCG
1201  AGACACAGTA CAACACTGTG AGTGACCACA TTAGCAAAGT CACTTTCTCC
1251  AACCTGAAAG CCACCAGACC TCGAGGGAAG TTTACCTATG ATGCAGTGTA
1301  CTGCTGCAAT GAGCAGGCAT GCCATCACCG CTACGCTGAA TTATATGTGA
```

FIG.2B

1351 TCGATGTCAA TATCAATATA TCATGTGAAA CTGACGGGTA CTTAACTAAA

1401 ATGACTTGCA GATGGTCACC CAGCACAATC CAATCACTAG TGGGAAGCAC

1451 TGTGCAGTTG AGGTATCACA GGCGCAGCCT GTACTGTCCC GATAATCCAT

1501 CTATTCGTCC TACATCAGAG CTCAAAAACT GCGTCTTACA GACAGATGGC

1551 CTTTATGAAT GTGTTTTCCA GCCAATCTTT CTATTATCTG GCTATACAAT

1601 GTGGATCAGG ATCAACCATT CTTTAGGTTC ACTTGACTCT CCACCAACGT

1651 GTGTCCTTCC TGACTCCGTA GTAAAACCAC TACCTCCATC TAATGTAAAA

1701 GCAGAGATTA CTATAAACAC TGGATTATTG AAAGTATCTT GGGAAAAGCC

1751 AGTCTTTCCA GAGAATAACC TTCAGTTCCA GATTCGATAT GGCTTAAATG

1801 GAAAAGAAAT ACAATGGAAG ACACACGAGG TATTCGATGC AAAATCAAAA

1851 TCGGCCAGCC TGCCAGTGTC AGATCTCTGT GCGGTCTATG TGGTACAGGT

1901 TCGCTGCCGG CGGTTGGATG GACTAGGGTA TTGGAGTAAT TGGAGCAGTC

1951 CAGCCTACAC TCTTGTCATG GATGTAAAAG TTCCTATGAG AGGGCCTGAA

FIG.2C

```
2001  TTCTGGAGAA TAATGGATGG GGATATTACT AAAAAGGAGA GAAATGTCAC
2051  CTTGCTTTGG AAGCCACTGA TGAAAAATGA CTCACTGTGT AGTGTGAGGA
2101  GGTATGTGGT GAAGCATCGT ACTGCCCACA ATGGGACATG GTCACAAGAT
2151  GTGGGAAATC AGACCAATCT CACTTTCCTG TGGGCAGAAT CAGCACACAC
2201  TGTTACAGTT CTGGCCATCA ATTCCATCGG TGCCTCCCTT GTGAATTTTA
2251  ACCTTACGTT CTCATGGCCC ATGAGTAAAG TGAATGCTGT GCAGTCACTC
2301  AGTGCTTATC CCCTGAGCAG CAGCTGCGTC ATCCTTTCCT GGACACTGTC
2351  ACCTAATGAT TATAGTCTGT TATATCTGGT TATTGAATGG AAGAACCTTA
2401  ATGATGATGA TGGAATGAAG TGGCTTAGAA TCCCTTCGAA TGTTAACAAG
2451  TATTATATCC ATGATAATTT TATTCCTATC GAGAAATATC AGTTTAGTCT
2501  TTACCCAGTA TTTATGGAAG GAGTTGGAAA ACCAAAGATA ATTAATGGTT
2551  TCACCAAAGA TGATATCGCC AAACAGCAAA ATGATGCAGG GCTGTATGTC
2601  ATTGTACCGA TAATTATTTC CTCTTGTGTC CTGCTGCTCG GAACACTGTT
2651  AATTTCACAC CAGAGAATGA AAAAGTTGTT TTGGGACGAT GTTCCAAACC
```

FIG.2D

```
2701  CCAAGAATTG TTCCTGGGCA CAAGGACTTA ATTTCCAAAA GCCTGAAACA
2751  TTTGAGCATC TTTTTACCAA GCATGCAGAA TCAGTGATAT TTGGTCCTCT
2801  TCTTCTGGAG CCTGAACCAG TTTCAGAAGA AATCAGTGTC GATACAGCTT
2851  GGAAAAATAA AGATGAGATG GTACCAGCAG CTATGGTCTC ACTTCTTTTG
2901  ACCACTCCAG ATTCCACAAG GGGTTCTATT TGTATCAGTG ACCAGTGTAA
2951  CAGTGCTAAC TTCTCTGGGG CTCAGAGCAC CCAGGGAACC TGTGAGGATG
3001  AGTGTCAGAG TCAACCCTCA GTTAAATATG CAACGCTGGT CAGCAACGTG
3051  AAAACAGTGG AAACTGATGA AGAGCAAGGG GCTATACATA GTTCTGTCAG
3101  CCAGTGCATC GCCAGGAAAC ATTCCCCACT GAGACAGTCT TTTTCTAGCA
3151  ACTCCTGGGA GATAGAGGCC CAGGCATTTT TCCTTTTATC AGATCATCCA
3201  CCCAATGTGA TTTCACCACA ACTTTCATTC TCAGGGTTGG ATGAGCTTTT
3251  GGAACTGGAG GGAAATTTTC CTGAAGAAAA TCACGGGGAA AAATCTGTGT
3301  ATTATCTAGG AGTCTCCTCA GGAAACAAAA GAGAGAATGA TATGCTTTTG
```

FIG.2E

3351 ACTGATGAGG CAGGGTATT GTGCCCATTC CCAGCTCACT GTCTGTTCAG

3401 TGACATCAGA ATCCTCCAGG AGAGTTGTTC ACACTTTGTA GAAAATAATT

3451 TGAATTTAGG GACCTCTGGT AAGAACTTTG TACCTTACAT GCCCCAGTTT

3501 CAATCCTGTT CCACTCACAG TCATAAGATA ATAGAAAATA AGATGTGTGA

3551 CTTAACTGTG TAATCTTGTC CAAAACTTC CAGGTTCCAT TCCAGTAGAG

3601 TGTGTCATGT ATAATATGTT CTTTTATAGT TGTGGGTGGG AGAGAAAGCC

FIG.2F

F=FORWARD R=REVERSE

PRIMER NAME	LOCATION IN RAT cDNA	OLIGO SEQUENCE	NOTES
ROBR 1	75-93 F	ATG ATG TG(C/T) CA(A/G) AA(A/G) TT(C/T) T	DEGENERATE TO MOUSE SEQUENCE
ROBR 2	108-127 F	CA(C/T) TGG GA(A/G) TT(C/T) CTI TA(C/T) GT	DEGENERATE TO MOUSE SEQUENCE
ROBR 3	462-478 F	GA(A/G) TG(T/C) TGG ATG AA(A/G) GG	DEGENERATE TO MOUSE SEQUENCE
ROBR 4	1158-1175 F	AA(A/G) CA(A/G) ATI GTI TGG TGG	DEGENERATE TO MOUSE SEQUENCE
ROBR 5	1590-1606 F	GGI TA(T/C) ACI ATG TGG AT	DEGENERATE TO MOUSE SEQUENCE
ROBR 6	1606-1590 R	ATC CAC ATI GT(A/G)TAI CC	DEGENERATE TO MOUSE SEQUENCE
ROBR 7	1945-1926 R	CTC CA(A/G) TT(A/G) CTC CA(A/G) TAI CC	DEGENERATE TO MOUSE SEQUENCE
ROBR 8	2282-2275 R	AC(T/C) TT(A/G) CTC ATI GGC CA	DEGENERATE TO MOUSE SEQUENCE
ROBR 9	2263-2045 R	CCA (T/C)TT CAT ICC (A/G)TC (A/G)TC	DEGENERATE TO MOUSE SEQUENCE
ROBR 10	133-153 F	CTG CAC TTA ACC TGG CCT ATC	RAT SPECIFIC PRIMER
ROBR 11	153-133 R	GAT AGG CCA GGT TAA GTG CAG	RAT SPECIFIC PRIMER
ROBR 12	380-361 R	GAG TGC GGA GCA GTT TTG AC	RAT SPECIFIC PRIMER
ROBR 13	930-951 F	GTC TCG GAT ACA TCT CTG CTG G	RAT SPECIFIC PRIMER
ROBR 14	1435-1427 R	GAT TGG ATT GTG CTG GGT G	RAT SPECIFIC PRIMER
ROBR 15	2047-2065 F	TCA CCT TGC TTT GGA AGC C	RAT SPECIFIC PRIMER
ROBR 16	2135-2155 F	GAC ATG GTC ACA AGA TGT GGG	RAT SPECIFIC PRIMER
ROBR 17	2216-2196 R	GGC CAG AAC TGT AAC AGT GTG	RAT SPECIFIC PRIMER
ROBR 18	435-455 F	CGC CAA CTA GGT GTA AAC TGG	RAT SPECIFIC PRIMER
ROBR 19	813-794 R	TGA CTT CCA TAC GCA AAC CC	RAT SPECIFIC PRIMER
ROBR 20	1444-1463 F	GAA GCA CTG TGC AGT TGA GG	RAT SPECIFIC PRIMER
ROBR 21	1815-1835 F	GGA AGA CAC ACG AGG TAT TCG	RAT SPECIFIC PRIMER
ROBR 22	673-693 F	CCA GAG CCA AAG TCA ACT ACG	RAT SPECIFIC PRIMER
ROBR 23	2338-2358 F	CCT GGA CAC TGT CAC CTG ATG	RAT SPECIFIC PRIMER
ROBR 24	R	CAT(T/C)TC (A/G)TC (T/C)TT (A/G)TT(T/C)TT CCA	DEGENERATE TO MOUSE C-TERMINUS

FIG.3A

ROBR 25	R	TC(A/G) CAC AT(T/C) TT(A/G) TT(T/C) TTC CA	DEGENERATE TO MOUSE C-TERMINUS
ROBR 26	R	AA(T/C) TGI GGC AT(A/G) TAI CC	DEGENERATE TO MOUSE C-TERMINUS
ROBR 27	796-816 F	GTT TGC GTA TGG AAG TCA CAG	RAT SPECIFIC PRIMER
ROBR 28	952-932 R	ACC AGC AGA GAT GTA TCC GAG	RAT SPECIFIC PRIMER
ROBR 29	2531-2548 F	CTG CTG CTC GGA ACA CTG	RAT SPECIFIC PRIMER
ROBR 30	2897-2874 R	AAG TGA GAC CAT AGC TGC TGG	RAT SPECIFIC PRIMER
ROBR 31	771-789 F	CTT CTG AAG CCC GAT CCA C	RAT SPECIFIC PRIMER
ROBR 33	R	GGG ACA AAA TTA CAC AGT TAA TTC ACA C	RAT SPECIFIC PRIMER
ROBR 34	2603-2583 R	AAT GAC ATA CAG CCC TGC ATC	RAT SPECIFIC PRIMER
ROBR 35	41-59 F	TTG GAG GAC TAT GGG TGT C	RAT SPECIFIC PRIMER
ROBR 36	3511-2493 R	GAA CAG GAT TGA AAG TGG G	RAT SPECIFIC PRIMER
ROBR 37	3598-3580 R	CTA CTG GAA TGG AAC CTG G	RAT SPECIFIC PRIMER
ROBR 38	646-666 F	GGG AAT GCG AAT GTC ATG TAC	RAT SPECIFIC PRIMER
ROBR 39	1014-995 R	AGC CAT CCA GTC TCT TGC TC	RAT SPECIFIC PRIMER
ROBR 40	1417-1435 F	CAC CCA GCA CAA TCC AAT C	RAT SPECIFIC PRIMER
ROBR 41	1793-1773 R	GCC ATA TCG AAT CTG GAA CTG	RAT SPECIFIC PRIMER
ROBR 42	2404-2424 F	ATG ATG ATG GAA TGA AGT GGC	RAT SPECIFIC PRIMER
ROBR 43	3110-3091 R	GAT GCA CTG GCT GAC AGA AC	RAT SPECIFIC PRIMER
ROBR 44	3091-3110 F	GTT CTG TCA GCC AGT GCA TC	RAT SPECIFIC PRIMER
ROBR 45	687-667 R	TGA CTT TGG CTC TGG GTA CTG	RAT SPECIFIC PRIMER
ROBR 46	2010-1991 R	TTC TCC AGA ATT CAG GCC CT	RAT SPECIFIC PRIMER
ROBR 47	2807-2826 F	GGA GCC TGA ACC AGT TTC AG	RAT SPECIFIC PRIMER
ROBR 48		TTT GAC TGA TGA GGC AGG G	RAT SPECIFIC PRIMER

FIG.3B

HOBR 1F	CTT ATG CTG GGA TGT GCC	HUMAN SPECIFIC 5' UTR PRIMER
HOBR 1F-2	TCG TGG CAT TAT CCT TCA G	HUMAN SPECIFIC 5' UTR PRIMER
HOBR 1R	TCT CTC CCA CCC ACA ACT AT	HUMAN SPECIFIC 3' UTR (OB-Rb) PRIMER
HOBR 5	CAT CAT (T/C)TC (A/G)TC (T/C)TT (A/G)TT	DEGENERATE TO HUMAN C-TERMINUS
HOBR 6	(T/C)TT CCA	DEGENERATE TO HUMAN C-TERMINUS
HOBR 7	GT(T/C) TG(A/G) AA(T/C) TGI GGC AT	DEGENERATE TO HUMAN C-TERMINUS
MOBR 1F	TC(A/G) CAC AT(T/C) TT(T/C) TCC AT	MOUSE SPECIFIC 5 UTR PRIMER
MOBR 1R	GTT CTG CAA ATC CAG GTG TA	MOUSE SPECIFIC 3' UTR (OB-Ro) PRIMER
	TGG GTT CAT CTG TAG TGG TC	

FIG.3C

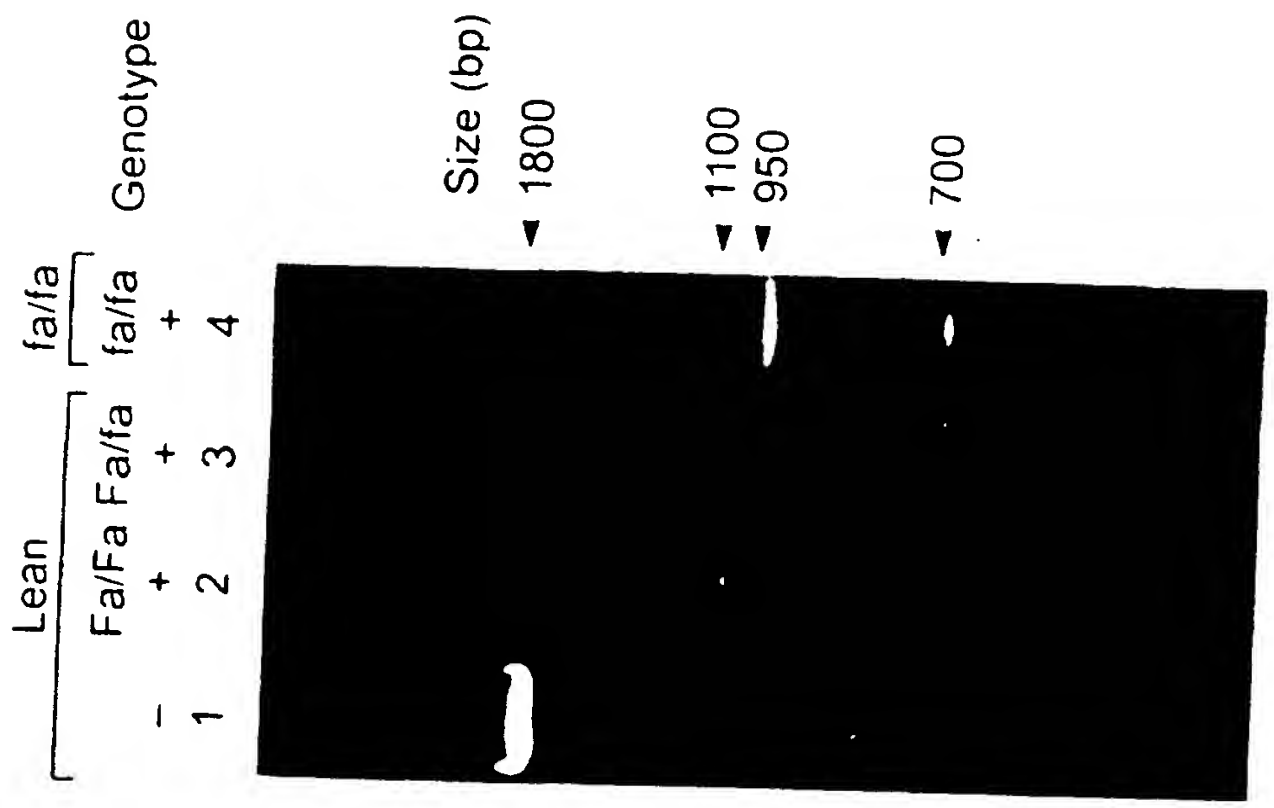
Genotype
fa/fa
fa/fa
+
4
Fa/fa
+
3
Fa/Fa
+
2
Lean
–
1
Size (bp)
1800
1100
950
700

FIG.4B

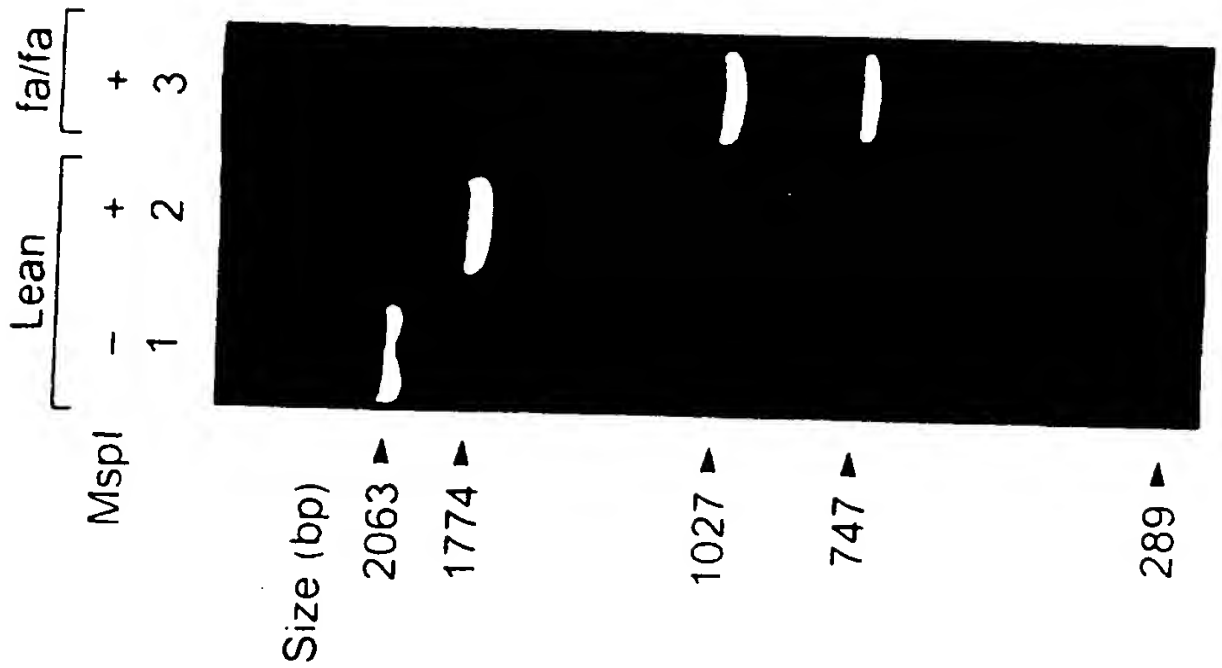
fa/fa
+
3
+
2
Lean
–
1
MspI
Size (bp)
2063
1774
1027
747
289

FIG.4A

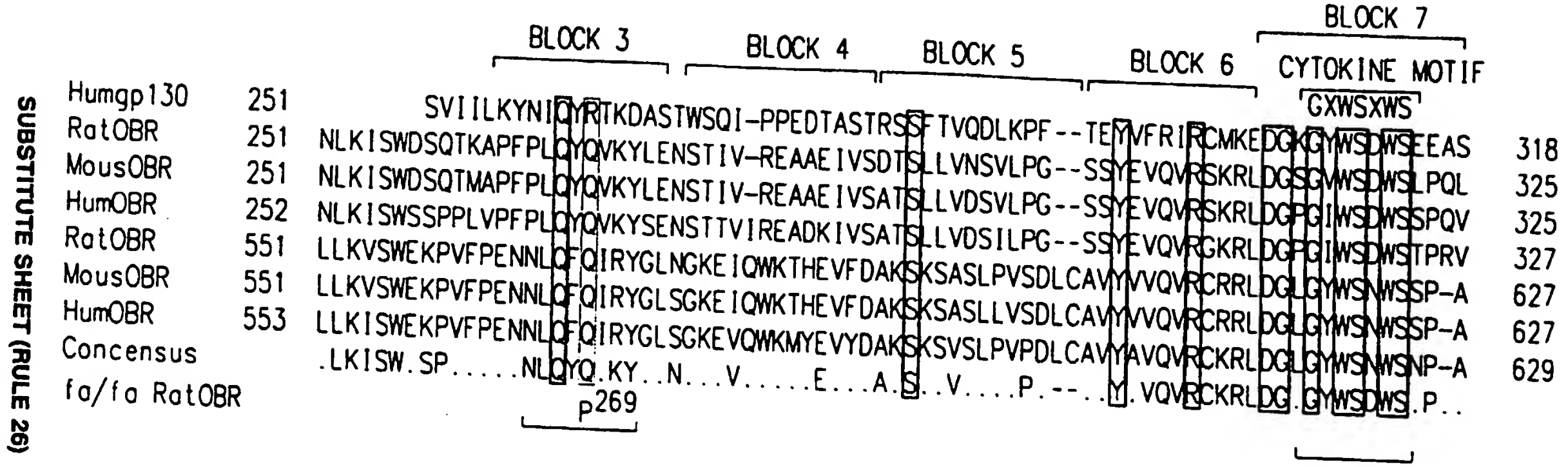
BLOCK 3
BLOCK 4
BLOCK 5
BLOCK 6
BLOCK 7
CYTOKINE MOTIF
GXWSXWS
P269
Humgp130 251 SVIILKYNIQYRTKDASTWSQI-PPEDTASTRSSFTVQDLKPF--TEYVFRIRCMKEDGKGYWSDWSEEAS 318
RatOBR 251 NLKISWDSQTKAPFPLQYQVKYLENSTIV-REAAEIVSDTSLLVNSVLPG--SSYEVQVRSKRLDGSGVWSDWSLPQL 325
MousOBR 251 NLKISWDSQTMAPFPLQYQVKYLENSTIV-REAAEIVSATSLLVDSVLPG--SSYEVQVRSKRLDGPGIWSDWSSPQV 325
HumOBR 252 NLKISWSSPPLVPFPLQYQVKYSENSTTVIREADKIVSATSLLVDSILPG--SSYEVQVRGKRLDGPGIWSDWSTPRV 327
RatOBR 551 LLKVSWEKPVFPENNLQFQIRYGLNGKEIQWKTHEVFDAKSKSASLPVSDLCAVYVVQVRCRRLDGLGYWSNWSSP-A 627
MousOBR 551 LLKVSWEKPVFPENNLQFQIRYGLSGKEIQWKTHEVFDAKSKSASLLVSDLCAVYVVQVRCRRLDGLGYWSNWSSP-A 627
HumOBR 553 LLKISWEKPVFPENNLQFQIRYGLSGKEVQWKMYEVYDAKSKSVSLPVPDLCAVYAVQVRCKRLDGLGYWSNWSNP-A 629
Concensus .LKISW.SP.....NLQYQ.KY..N...V.....E...A.S..V....P.--..Y.VQVRCKRLDG.GYWSDWS.P..
fa/fa RatOBR

FIG.5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02397

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/300; 536/23.1, 24.3, 24.33; 435/320.1, 254.2, 240.2, 252.3, 91.2, 6 , 172.3, 69.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	Tartaglia et al. Identification and Expression Cloning of a Leptin Receptor, OB-R. Cell. 29 December 1995, Volume 83, pages 1263-1271, see entire document.	1-7,10-15, 17-20 ---------- 8,9,16
X,P	Phillips et al. Leptin receptor missense mutation in the fatty Zucker rat. Nature Genetics. May 1996, Volume 13, No. 1, pages 18-19, see entire document.	1-20
Y	Murakami et al. Cloning of Rat OBESE cDNA and its Expression in Obese Rats. Biochemical and Biophysical Research Communications. 26 April 1995, Volume 209, Number 3, pages 944-952. see entire document.	20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 28 MARCH 1997	Date of mailing of the international search report 22 MAY 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DIANNE REES Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02397

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07K 5/00; C07H 21/02, 21/04; C12N 15/70, 5/10, 1/19, 1/21, 15/63; C12P 19/34, 21/00; C12Q 1/68

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

530/300; 536/23.1, 24.3, 24.33; 435/320.1, 254.2, 240.2, 252.3, 91.2, 6 , 172.3, 69.1

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, BIOSIS, BIOTECHABS, BIOTECHDS, CANCERLIT, CABA, GENBANK, EMBASE, SCISEARCH, CANCERLIT, MEDLINE, TOXLINE, TOXL IT, DRUGU, SCISEARCH, DISSABS, USPATFULL, JAPIO, INPADOC, WPIDS
search terms: obestity, fa, ob, leptin, OB-R, leptin receptor.